Beatrice Hill virus represents a novel species in the genus *Tibrovirus* (*Mononegavirales*: *Rhabdoviridae*)

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ABSTRACT

The rhabdoviral genus *Tibrovirus* currently has three official members assigned to two species: Bivens Arm virus and Tibrogargan virus (species *Tibrogargan tibrovirus*) and Coastal Plains virus (species *Coastal Plains tibrovirus*). Here we report the complete genome sequence of a new putative member of this genus, Beatrice Hill virus. Although relatively closely related to the three classified viruses, Beatrice Hill virus represents a novel tibrovirus species.

GENOME ANNOUNCEMENT

The mononegaviral family *Rhabdoviridae* currently includes 13 genera and four unassigned species (1). One of these genera, *Tibrovirus*, includes two species. Bivens Arm virus (BAV) and Tibrogargan virus (TIBV) have been assigned to the species *Tibrogargan tibrovirus*, and Coastal Plains virus (CPV) has been assigned to the species *Coastal Plains tibrovirus* (1-3). TIBV and BAV were originally isolated from biting midges (*Culicoides brevitarsis* and *Culicoides insignis*, respectively), and CPV from an apparently health steer (4-6). Recently, several novel viruses have been identified as putative tibroviruses. These include: 1) Bas-Congo virus (BASV) in the serum of a human with viral hemorrhagic fever (7), 2) Ekpoma virus 1 (EKV-1) and Ekpoma virus 2 (EKV-2) in sera of apparently healthy humans (8), and Sweetwater Branch virus (SWBV) in biting midges (*Culicoides insignis*) (3, 6). These viruses have recently been accepted by the International Committee on Taxonomy of Viruses (ICTV) to represent the novel species *Bas Congo tibrovirus*, *Ekpoma 1 tibrovirus*, *Ekpoma 2 tibrovirus*, and *Sweetwater Branch tibrovirus*, respectively (9). All tibroviruses have the specific genomic structure 3′-N-P-M-U1-U2-G-U3-L-5′, with N-P-M-G-L being canonical rhabdoviral genes encoding structural proteins U1–U3 being tibrovirus-unique genes. U1 and U2 encode proteins of unknown function and U3 encodes a protein with the structural characteristics of a viroporin (3, 9, 10).

Beatrice Hill virus (BHV) was first reported in 1984 as a novel virus of biting midges (*Culicoides peregrinus*) that had been collected at Beatrice Hill, Northern Territory, Australia (11). In 2016, Huang *et al*. published a 5,734 nt-long contig of the Beatrice Hill virus genome, which indicated that this virus most likely falls into the tibrovirus clade (12). To determine the taxonomic position of BHV, we obtained a historical sample of brain tissue obtained from a laboratory mouse that had been infected intracranially with the BHV isolate CSIRO25 10 (Commonwealth Scientific and Industrial Research Organisation, Geelong, Australia). Viral RNA from the sample was processed and sequenced to obtain the complete genome using a sequence-independent single-primer amplification (SISPA) protocol that included rapid amplification of cDNA ends (RACE) (13). Resulting libraries were sequenced on an Illumina MiSeq Desktop sequencer. Illumina and SISPA-RACE adapter sequences were trimmed from the sequencing reads using Cutadapt-1.2.1 (14), quality filtering was conducted with Prinseq-lite (15) and reads were assembled into contigs using Ray Meta with kmer length = 25 (16). Resultant contigs were aligned to the NCBI sequence database using BLAST. Reads were aligned back to the assembled BHV sequence using Bowtie2 (17) and custom scripts to generate a final consensus sequence.

Using PASC (18), the determined complete BHV genome sequence was found to be ≈72.79% identical to that of TIBV (GenBank #GQ294472.1) and 72.48% identical to that of BAV (KP688373.1). The genome organization matches that of all tibroviruses. We therefore confirm Huang *et al*.'s hypothesis that BHV is a tibrovirus, and suggest that this virus be assigned to a novel species ("*Beatrice Hill tibrovirus*") based on its divergence from TIBV and BAV.

Nucleotide sequence accession numbers. The GenBank accession number of Beatrice Hill virus isolate CSIRO25 10 is xxx.

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